AMENDMENT

In the Specification:

Please replace the paragraph beginning on page 32, line 16 and ending on page 33, line 10 with the following amended paragraph:

In one preferred embodiment, the isolated polynucleotide comprises a nucleotide sequence encoding the polypeptide comprising SEQ ID NO: 2. Importantly, in contrast to the published sequence of Langmann et al. which codes for a protein of 2201 amino acids based on a predicted start methionine found in exon 3 (Langmann et al., *Biochem. Biophys. Res. Comm.*, 257: 29-33 (1999) (GenBank Accession No. AJO12376), the presently claimed nucleotide sequence contains 50 exons and codes for a protein of 2261 amino acids (see Figure 4). The corresponding nucleotide sequence of the present invention contains a coding sequence that includes an additional 180 nucleotides at the 5' end corresponding to the following 60 amino-terminal amino acids: MACWPQLRLLLWKNLTFRRRQTCQLLLEVAWPLFIFLILISVRLSYPPYEQHECHFPNKA (SEQ ID NO: 58). Given that there is an in-frame stop codon 6 to 9 nucleotides upstream from this location, the newly predicted start site is the first methionine codon that could produce a continuous open reading frame. Alignment of this new ABC1 cDNA sequence with related ABC transporter sequences ABCR and ABC-C (also known as ABC3) which also contain open reading frames for the 60 additional amino acids, indicates a high degree of similarity, implying that the homologous ABC transporter proteins begin with sequences related to the amino terminal extension sequence proposed for human ABC1. It is likely that the earlier published start site of the human ABC1 was predicted from the published mouse ABC1 cDNA sequence (Luciani et al., *Genomics,* 21150-159 (1994); GenBank Accession no.: X75926) which contains an extra nucleotide "n" in the extension region such that the newly disclosed methionine is not in-frame. However, if the "n" nucleotide in the

McDONNELL BOEHNEN,
HULBERT & BERGHOFF
300 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606
TELEPHONE (312) 913-0001
FACSIMILE (312) 913-0002

mouse sequence is ignored, the mouse and human sequences of the extension region are identical. In light of these results, it is likely that the full length human ABC1 protein contains 2261 amino acids rather than 2201 amino acids, as previously suggested by Langmann et al. and others. Accordingly, Langmann et al. do not present the full open reading frame of human ABC1.

Please replace the paragraph beginning on page 35, line 24 and ending on page 36, line 12 with the following amended paragraph:

Another aspect of the present invention relates to isolated polynucleotides that comprise the non-coding 5' flanking and 3' flanking regions of ABC1. In one embodiment, the isolated polynucleotide comprises the non-coding 5' flanking region of ABC1. Preferably, the 5' flanking region contains, but is not limited to, the ABC1 promoter region. Thus, in a preferred embodiment, the polynucleotide comprises the sequence shown in SEQ ID NO: 3. As demonstrated by heterologous reporter assays, discussed in Example 15, the polynucleotide set forth in SEQ ID NO: 3 contains the transcriptional regulatory region of the ABC1 gene. As shown in Figure 13, the polynucleotide set forth in SEQ ID NO: 3 is a 1643 b.p. non-coding sequence that contains several transcription regulatory elements, including a TATA box at positions 1522, 1435, and 1383, as well as transcription factor binding sites, including several putative SP1 sites, and several nuclear receptor half sites. In addition, an identified sterol response element is found at position 1483-1500. Further heterologous reporter assays described in Example ~~17~~ 18 revealed that several discrete portions of SEQ ID NO: 3 retained promoter activity. Accordingly, in another preferred embodiment, the polynucleotide comprises nucleotides 1-1532, 1080-1643, 1181-1643, 1292-1643, or 1394-1643 of SEQ ID NO: 3. In an especially preferred embodiment, the polynucleotide comprises nucleotides 1394-1532 of SEQ ID NO: 3, which sequence has been shown to have ABC1 promoter activity (see Example ~~17~~ 18). In yet another preferred embodiment, the polynucleotide comprises nucleotides 1480-1510

McDONNELL BOEHNEN,
HULBERT & BERGHOFF
300 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606
TELEPHONE (312) 913-0001
FACSIMILE (312) 913-0002

of SEQ ID NO: 3, which is shown to regulate the ABC1 transcriptional response to LXR ligands.

Please replace the paragraph beginning on page 36, line 19 and ending on page 37, line 5 with the following amended paragraph:

In another embodiment, the isolated polynucleotide comprises the 3' flanking region of ABC1. Several 3' untranslated regions have been identified which may represent alternate sites of polyadenylation of the ABC1 transcript. Preferably, the 3' flanking region contains regulatory sequences. For example, the full length 3' UTR (SEQ ID NO: 6) contains 46 sequences (AA)nCU/UC(AA)n (SEQ ID NO: 59) which have been shown to be necessary for binding of Vigilin. Vigilin, a ubiquitous protein with 14K homology domains, is the estrogen-inducible vitellogenin mRNA 3'-untranslated region binding protein (*J. Biol. Chem.*, 272: 12249-12252 (1997)). In addition to binding HDL, Vigilin has been shown to bind to the 3' flanking region of mRNAs and to increase the half-life of the mRNA transcript (*Mol. Cell. Biol.*, 18:3991-4003 (1998)). Thus, the 3' flanking region could be altered, for example, to increase the binding of Vigilin, thereby increasing the half-life of the ABC1 mRNA. Preferably, the isolated polynucleotide comprises the sequence shown in SEQ ID NO: 4. Also preferably, the isolated polynucleotide comprises the sequence shown in SEQ ID NO: 5. In another preferred embodiment, the isolated polynucleotide comprises the sequence shown in SEQ ID NO: 6. In other preferred embodiments, the polynucleotide comprises a sequence that hybridizes, under stringent conditions, to the nucleotide sequence set forth in SEQ ID NO: 4, SEQ ID NO: 5, or SEQ ID NO: 6.

McDONNELL BOEHNEN,
HULBERT & BERGHOFF
300 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606
TELEPHONE (312) 913-0001
FACSIMILE (312) 913-0002

Please replace the paragraph on page 58, lines 10-23 with the following amended paragraph:

The dosage regimen for treating a cardiovascular disease with a composition comprising an ABC1 polynucleotide or ABC1 expression vector is based on a variety of factors, including the type of cardiovascular disease, the age, weight, sex, medical condition of the patient, the severity of the condition, the route of administration, and the particular compound employed. Thus, the dosage regimen may vary widely, but can be determined routinely using standard methods. For example, the amount of ABC1 polynucleotide or ABC1 expression vector to be administered is an amount sufficient to increase cholesterol efflux from the cells of a mammalian subject. Such amount can be determined, for example, by measuring the plasma HDL-cholesterol level of a subject before and after administration of the ABC1 polynucleotide or ABC1 expression vector. A sufficient amount of ABC1 polynucleotide or ABC1 expression vector is an amount that increases the plasma HDL-cholesterol level of a subject. Accordingly, the clinician can titer the dosage and modify the route of administration to obtain the optimal therapeutic effect. A typical dosage may range from about 0.1 ~~μ~~g/kg to about 100 mg/kg or more, depending on the factors mentioned above.

Please replace the paragraph beginning on page 75, line 28 and ending on page 76, line 10 with the following amended paragraph:

The amount of ABC1 protein can be assayed using any of the well-known methods of measuring protein. Preferably, the amount of ABC1 protein is measured using an immunoassay. In one embodiment, the amount of ABC1 protein is determined by (a) contacting the cell sample with a population of anti-ABC1 antibodies and (b) detecting the anti-ABC1 antibodies associated with the cell sample. For example, the ABC1 protein can be contacted with an antiserum raised against a synthetic peptide corresponding to KNQTVVDAVLTSFLQDEKVKES (SEQ ID NO: 60) located at the C-terminus, as described in Example 11. The anti-ABC1 antibodies can be detected using several methods known in the art, including, for example, western blotting, immunoprecipitation, and FACS, wherein the detection can be accomplished using radioactive, colorometric, or

McDONNELL BOEHNEN,
HULBERT & BERGHOFF
300 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606
TELEPHONE (312) 913-0001
FACSIMILE (312) 913-0002

fluorescent labeling. One preferred method for measuring the amount of ABC1 protein in a cell sample is immunoprecipitation, wherein biotinylated ABC1 proteins are contacted with anti-ABC1 antibody and the bound anti-ABC1 antibody is detected using streptavidin horse radish peroxidase.

Please replace the paragraph on page 89, lines 1-20 with the following amended paragraph:

Previous genetic linkage analysis mapped the TD gene to the 7-cM region of human chromosome 9q31 (Rust et al., *Nat. Genet.*, 20, 96-98 (1998)). In addition, *in situ* hybridization analyses revealed that the ABC1 gene was localized to the broader chromosomal interval 9q22 - 9q31 (Luciani et al., *Genomics*, 21, 150-159 (1994)). Using PCR methods with the GeneBridge 4 panel of human/hampster radiation hybrids (Research Genetics, Inc., Huntsville, AL), human ABC1 was determined to be located between the markers WI-14706 and WI-4062, corresponding to the 7-cM region of human chromosome 9q31. DNA from 93 human/hampster hybrid cell lines was amplified by PCR using human ABC1-specific primers LF: CCTCTCATTACACAAAAACCAGAC (SEQ ID NO: 11) and LR: GCTTTCTTTCACTTCTCATCCTG (SEQ ID NO: 12). Each line was scored as positive or negative for the human ABC1 amplification product and the mapping of ABC1 derived from analysis of this data was accomplished using the Whitehead Institute/MIT Center for Genome Research software, accessed via the internet ~~(http://carbon.wi.mit.edu:8000/cgi-bin/contig/rhmapper.pl)~~ (http://carbon.wi.mit.edu:8000/cgi-bin/contig/rhmapper.pl). These results were further confirmed by southern blot hybridization to human genomic/yeast artificial chromosome clones (Research Genetics, Inc.) from the equivalent interval. In addition, public database searching (GeneMap '98; National Center for Biotechnology Information) and radiation hybrid mapping eliminated the other significantly underexpressed genes in the microarray data from the location in the reported genetic interval. These complementary data demonstrate that the ABC1 gene is located on human chromosome 9q31 and further indicate that the ABC1 gene is associated with Tangier disease.

McDONNELL BOEHNEN,
HULBERT & BERGHOFF
300 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606
TELEPHONE (312) 913-0001
FACSIMILE (312) 913-0002

Please replace the paragraph on page 99, lines 20-28 with the following amended paragraph:

Immunoprecipitation: Rabbit antiserum for ABC1 was raised against a synthetic peptide corresponding to the deduced peptide KNQTVVDAVLTSFLQDEKVKES (SEQ ID NO: 60) located at the C-terminus of human ABC1. Immunoprecipitation was performed by solubilizing the cells in PBS containing 1% Triton X-100 (Sigma, St. Louis, MO) and protease inhibitors leupeptin (1mM), pepstatin (1mM), and aprotinin (1mM). The cell extract was incubated overnight at 4°C with anti-ABC1 antiserum at 1:200 dilution followed by an additional 1 hour incubation with 5µl proteinA-coated magnetic beads (Dynal, Lake Success, NY; Cat. #1001.01). The antibody-antigen complex was sedimented with a magnet, the beads were washed twice with 1% Triton-X/PBS, and the proteins were eluted with 1% acetic acid.

Please replace the paragraph on page 107, lines 16-22 with the following amended paragraph:

To determine which portion of the 5' flanking region of ABC1 retains transcriptional activity in response to nuclear ligands, various plasmids containing a different portion of the 5' flanking region and a luciferase reporter gene were transfected into RAW 264.7 cells treated with at least one ligand for the nuclear receptors. Using this system, ~~an~~ a sterol response element corresponding to nucleotides 1480-1510 of SEQ ID NO: 3 was identified. The sterol response element contains a direct repeat-4 element TGACCGatagTAACCT (SEQ ID NO: 61). Confirmation of the sterol response element was obtained using site-directed mutagenesis and band-shift assay techniques.

McDONNELL BOEHNEN,
HULBERT & BERGHOFF
300 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606
TELEPHONE (312) 913-0001
FACSIMILE (312) 913-0002

Please replace the paragraph on page 108, lines 18-22 with the following amended paragraph:

Site-Directed Mutagenesis: The sterol response element corresponding to nucleotides 1480-1510 of SEQ ID NO: 3 was mutated in the 1080-1643 sequence described above using site-directed mutagenesis. Specifically, the response element containing a direct repeat-4 element TGACCGatagTAACCT (SEQ ID NO: 61) was mutated to CTGCACatagTAACCT (SEQ ID NO: 62) using the GeneEditor system (Promega, Madison, WI) according to the manufacturer's protocol.

McDONNELL BOEHNEN,
HULBERT & BERGHOFF
300 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606
TELEPHONE (312) 913-0001
FACSIMILE (312) 913-0002